Figure ~~16~~ 7

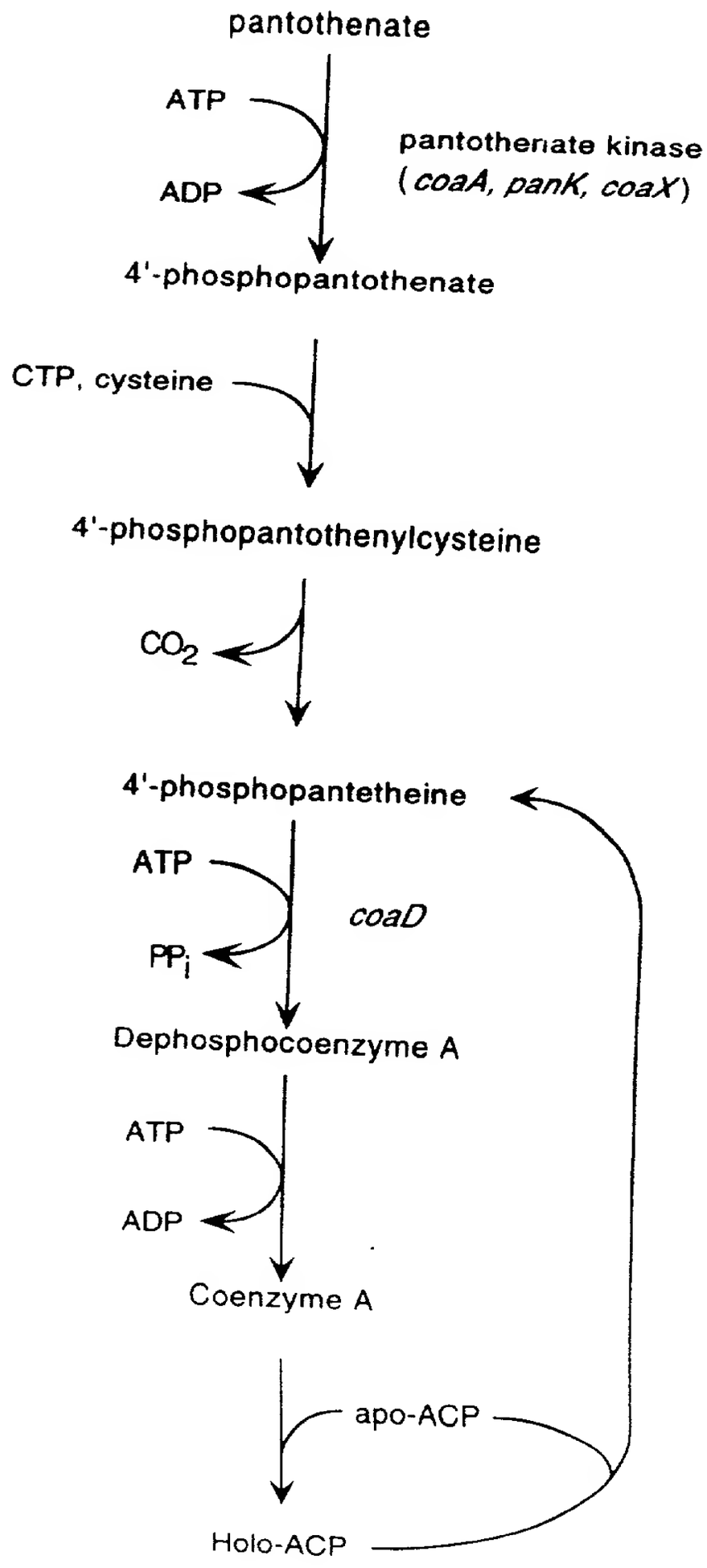
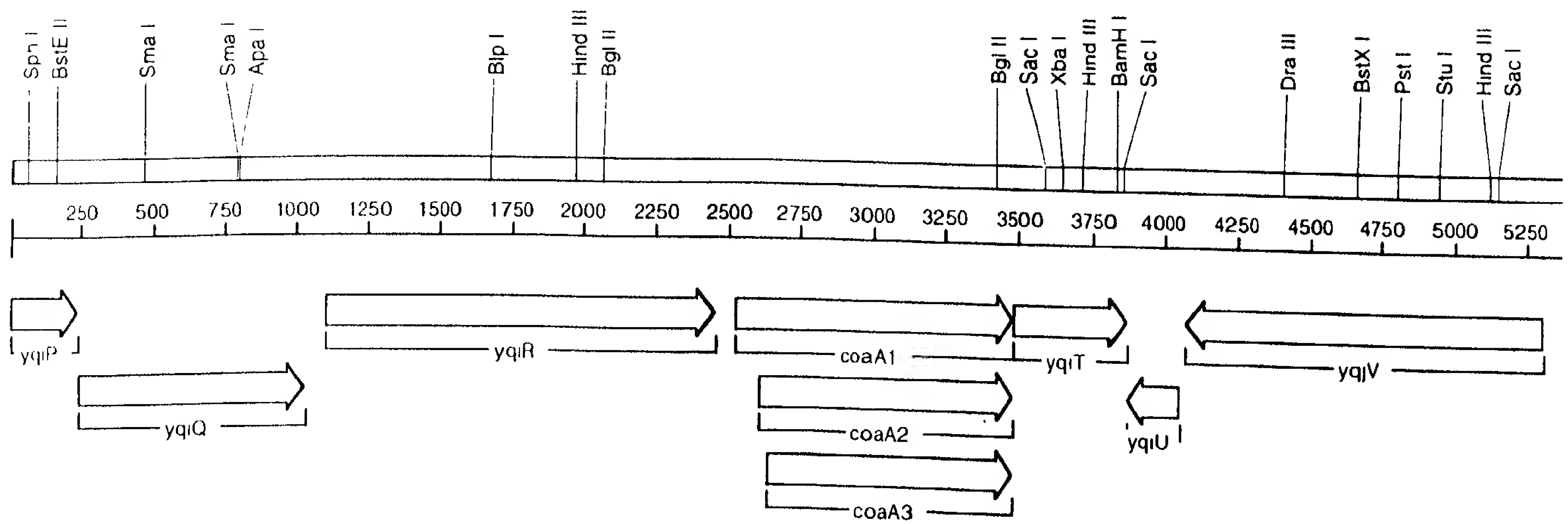

pantothenate
ATP
ADP
pantothenate kinase
(coaA, panK, coaX)
4'-phosphopantothenate
CTP, cysteine
4'-phosphopantothenylcysteine
CO2
4'-phosphopantetheine
ATP
PPi
coaD
Dephosphocoenzyme A
ATP
ADP
Coenzyme A
apo-ACP
Holo-ACP

Figure 2 Structure of the B. subtilis chromosome in the region of the coaA gene. The scale is in base pairs and the significant open reading frames are shown by the open arrows.



B

Figure 1: Structure of pAN296, designed to delete most of the **B. subtilis coaA** *gene and substitute a chloramphenicol resistance gene.*

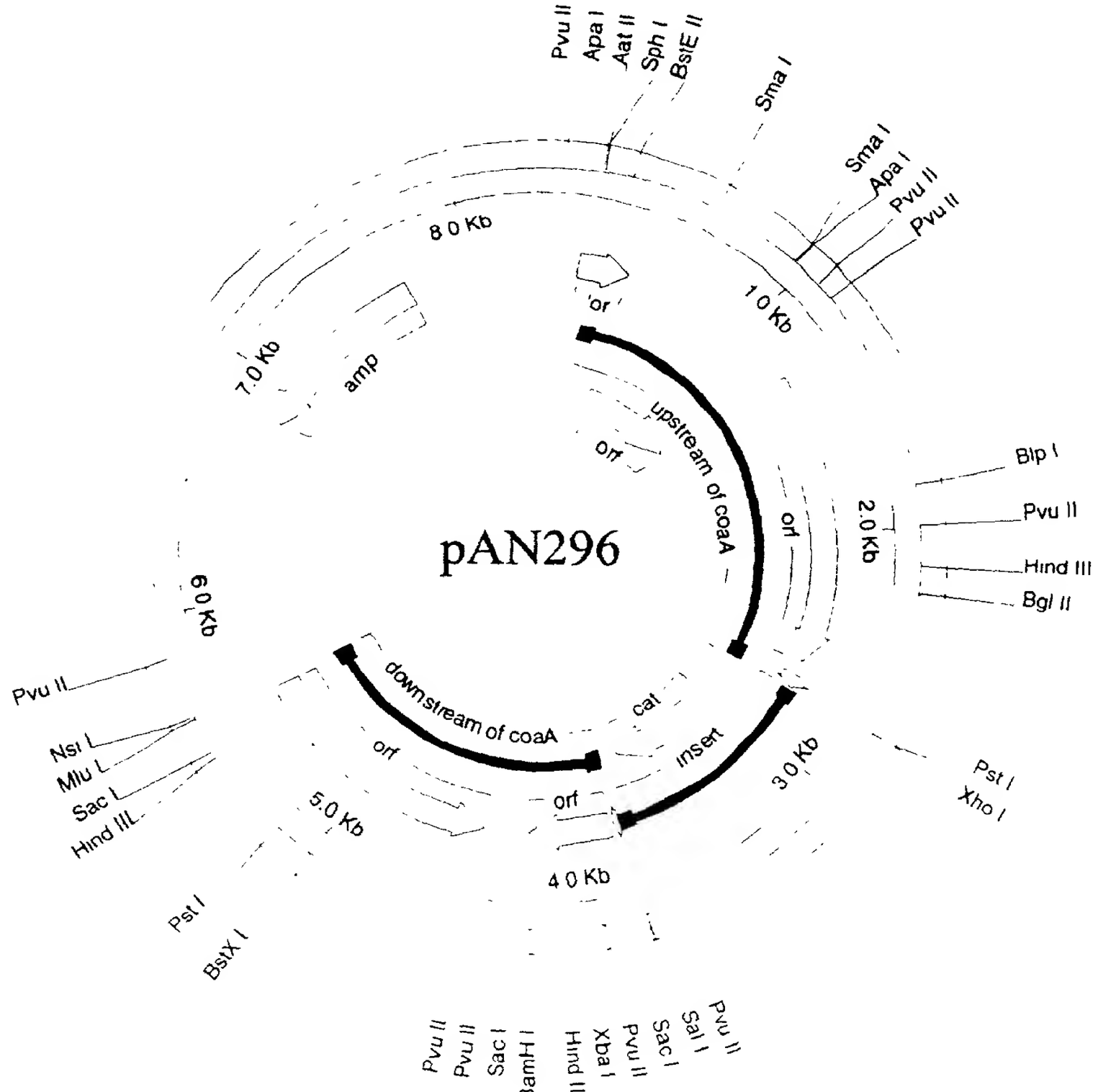
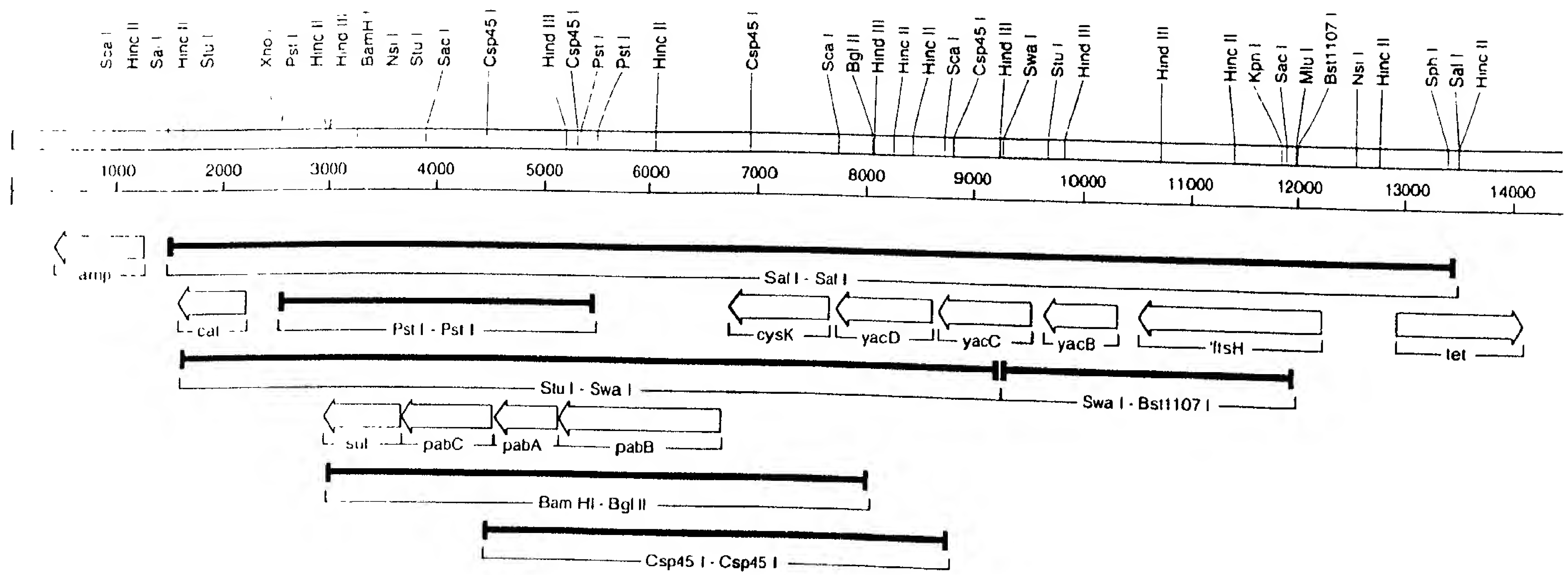
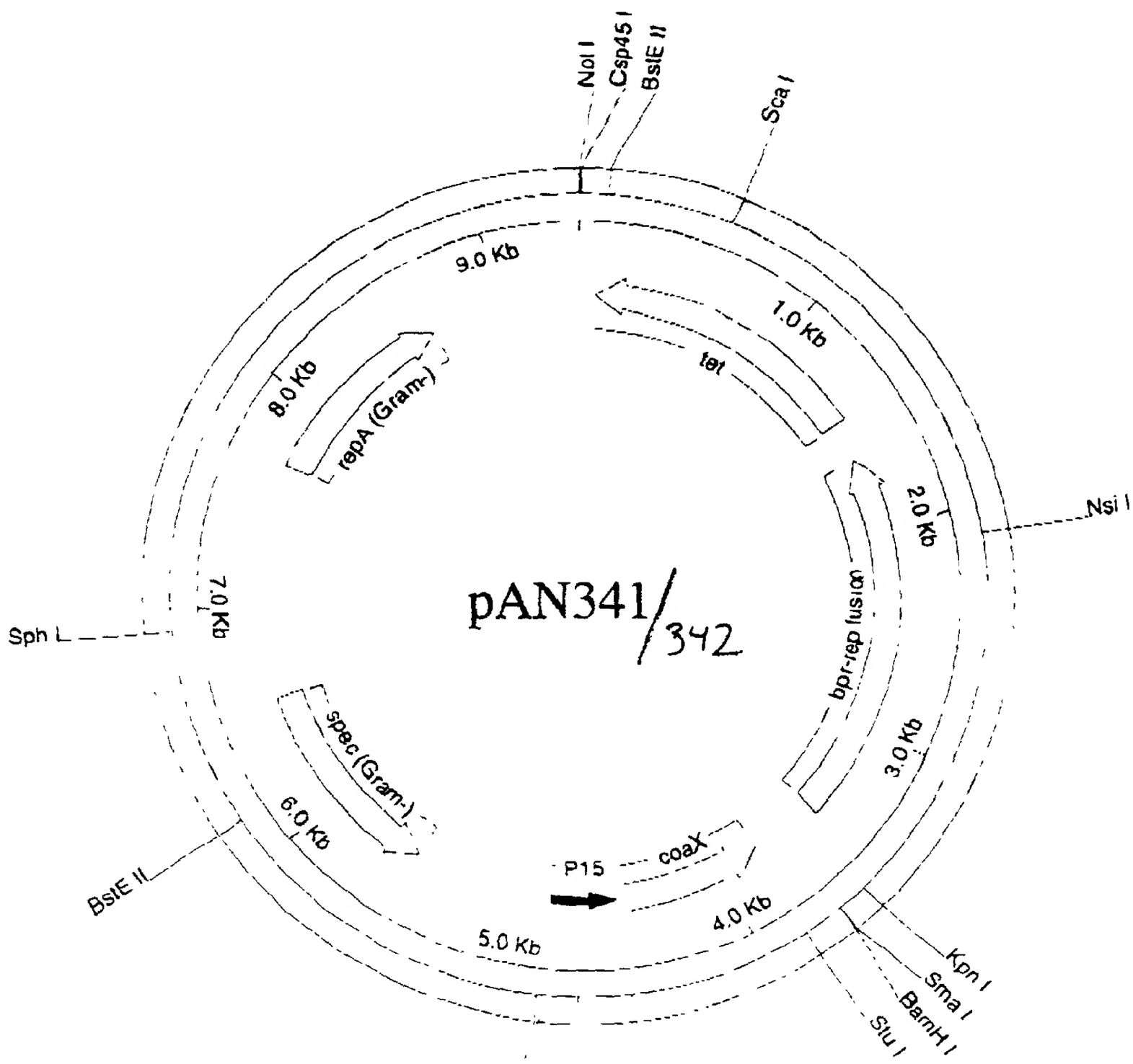


Figure ~~21~~ 4


Sca I
Hinc II
Sal I
Hinc II
Stu I
Xho I
Pst I
Hinc II
Hind III
BamH I
Nsi I
Stu I
Sac I
Csp45 I
Hind III
Csp45 I
Pst I
Pst I
Hinc II
Csp45 I
Sca I
Bgl II
Hind III
Hinc II
Hinc II
Sca I
Csp45 I
Hind III
Swa I
Stu I
Hind III
Hind III
Hinc II
Kpn I
Sac I
Mlu I
Bst1107 I
Nsi I
Hinc II
Sph I
Sal I
Hinc II
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
11000
12000
13000
14000
amp
Sal I - Sal I
cat
Pst I - Pst I
cysK
yacD
yacC
yacB
'ftsH
tet
Stu I - Swa I
Swa I - Bst1107 I
sul
pabC
pabA
pabB
Bam HI - Bgl II
Csp45 I - Csp45 I

5

Figure 22 Structure of pAN341and pAN342, two independent PCR- derived clones of **yacB** *(renamed* **coaX***)* .



Not I
Sal I
Xho I
Xba I

6A

FIG. 23A

```
CLUSTAL W (1.7) Multiple Sequence Alignments

Sequence type explicitly set to Protein
Sequence format is Pearson
Seq  1: B.subtilis|CoaX|SEQNO 2  258 aa   Seq.8: sp|O51477|B.burgdorferi        262 aa
Seq.2: dbj|BAA21476.1|D.vulgaris 212 aa   Seq.9: sp|P74045|Synechocystis       257 aa
Seq.3: gb|AAD35964.1|T.maritima  246 aa   Seq.10: sp|O25533|H.pylori           223 aa
Seq.4: pir|T36391|S.coelicolor   265 aa   Seq.11: sp|O67753|A.aeolicus         229 aa
Seq.5: sp|Q45338|B.pertussis     267 aa   Seq.12: sp|Q9RX54|D.radiodurans      262 aa
Seq.6: sp|O06282|M.tuberculosis  272 aa   Seq.13: WIT|RCA03301|C.acetobutylicum 250 aa
Seq 7: sp|O83446|T.pallidum      273 aa   Seq.14: WIT|RRC02473|R.capsulatus    258 aa

B.subtilis|CoaX|SEQIDNO 2      ----------------MLLVIDVGNTNTVLGVYHDG----KLEYHWRIE
WIT|RCA03301|C.acetobutylicum  NKRAAFMLLLFLRSVLKVILVLDVGNTNIVLGIYNDT----KLTAEWRLS
pir|T36391|S.coelicolor        ----------------MLLTIDVGNTHTVLGLFDGE----DIVEHWRIS
sp|O06282|M.tuberculosis       ----------------MLLAIDVRNTHTVVGLLSGMKEHAKVVQQWRIR
WIT|RRC02473|R.capsulatus      ----------------MLLCIDCGNTNTVFSVWDGT----DFAATWRIA
dbj|BAA21476.1|D.vulgaris      --------------MTQHFLLFDIGNTNVKIGIAVET----AVLTSYVLP
sp|Q9RX54|D.radiodurans        -------------MPAFPLLAVDIGNTTTVLGLADASG---ALTHTWRIR
gb|AAD35964.1|T maritima       ----------------MYLLVDVGNTHSVFSITEDG----KTFRRWRLS
sp|O83446|T.pallidum           ----------------MLLIDVGNSHVVFGIQGENGGRVCVRELFRLA
sp|O51477|B.burgdorferi        ----------MNKPLLSELIIDIGNTSIAFALFKDN----QVNLFIKMK
sp|O67753|A.aeolicus           ---------------MRFLTVDVGNSSVDIALWEGK----------KVK
sp|P74045|Synechocystis        ----------METSKPGCGLALDNDKQKPWLGLMIGN-----SRLHWAYC
sp|O25533|H.pylori             -------------MPARQSFTDLKN---LVLCDIGN----------TR
sp|Q45338|B.pertussis          ----------------MIILIDSGNSRLKVGWFDPDAP--QAAREPAPV
                                                *  :    .
```

6B

FIG. ~~23B~~ 2

```
B.subtilis|CoaX|SEQIDNO_2      TSRHKTEDEFGMILRSLFDHS----GLMFEQIDGIIISSVVPPIMFALER
WIT|RCA03301|C.acetobutylicum  TDVLRSADEYGIQVMNLFQQD----KLDPTLVEGVIISSVVPNIMYSLEH
pir|T36391|S.coelicolor        TDSRRTADELAVLLQGLMGMHPLLGDELGDGIDGIAICATVPSVLHELRE
sp|O06282|M.tuberculosis       TESEVTADELALTIDGLIG-------EDSERLTGTAALSTVPSVLHEVRI
WIT|RRC02473|R.capsulatus      TDHRRTADEYFVWLNTLMQLK-----GLQGRISEAIISSTAPRVVFNLRV
dbj|BAA21476.1|D.vulgaris      TDPGQTTDSIGLRLLEVLRHAG----LGPADVGACVASSVVPGVNPLIRR
sp|Q9RX54|D.radiodurans        TNREMLPDDLALQLHGLFTLA-----GAP-IPRAAVLSSVAPPVGENYAL
gb|AAD35964.1|T.maritima       TGVFQTEDELFSHLHPLLG-------DAMREIKGIGVASVVPTQNTVIER
sp|O83446|T.pallidum           PDARKTQDEYSLLIHALCERAG----VGRASLRDAFISSVVPVLTKTIAD
sp|O51477|B.burgdorferi        TNLMLRYDEVYSFFEENFDFN------VN---K-VFISSVVPILNETFKN
sp|O67753|A.aeolicus           DFLKLSHEEFLKEEFPKLK--------------ALGISVKQSFSEKVRG
sp|P74045|Synechocystis        SGNAPLQTWVTDYNPKSAQLP-------------VLLGKVPLMLASVVPE
sp|O25533|H pylori             IHFAQNYQLFSSAKEDLKR---------------LGIQKEIFYISVNEE
sp|Q45338|B.pertussis          AFDNLDLDALGRWLATLPRRP-------Q----RALGVNVAGLARGEAIA

B.subtilis|CoaX|SEQIDNO_2      MCTKYFHIEPQIVG-PG-MKTGLNIKYDNPKEVGADRIVNAVAAIHLYG-
WIT|RCA03301|C.acetobutylicum  MIRKYFKINPLVVG-PG-IKTGINIKYDNPKEVGADRIVNAVAAHEIYK-
pir|T36391|S.coelicolor        VTRRYYGDVPAVLVEPG-VKTGVPILTDHPKEVGADRIINAVAAVELYG-
sp|O06282|M.tuberculosis       MLDQYWPSVPHVLIEPG-VRTGIPLLVDNPKEVGADRIVNCLAAYDRFR-
WIT|RRC02473|R.capsulatus      LCNRYFDCRPYVVGKPG-CELPVAPRVDPGTTVGPDRLVNTVAGYDRHG-
dbj|BAA21476.1|D.vulgaris      ACERYL--YRKLLFAPGDIAIPLDNRYERPAEVGADRLVAAYAARRLYP-
sp|Q9RX54|D.radiodurans        ALKRHFMIDAFAVSAEN--LPDVTVELDTPGSVGADRLCNLFGAEKYLG-
gb|AAD35964.1|T.maritima       FSQKYFHISPIWVKAKN---GCVKWNVKNPSEVGADRVANVVAFVKEYG-
sp|O83446|T.pallidum           AVAQISGVQPVVFGPWAYEHLPVRIPEPVRAEIGTDLVANAVAAYVHFR-
sp|O51477|B.burgdorferi        VIFSFFKIKPLFIGFDLNYDLTFNPYKSDKFLLGSDVFANLVAAIENYS-
sp|O67753|A.aeolicus           KIPKIK-----FLKKEN---FPIQVDYKTPETLGTDRVALAYSAKKFYG-
sp|P74045|Synechocystis        QTEVWRVYQPKILTLKN---LPLVNLYP---SFGIDRALAGLGTGLTYG-
sp|O25533|H.pylori             NEKALLNCYPNAKNIAG--FFHLETDYVG---LGIDRQMACLA---VN--
sp|Q45338|B.pertussis          ATLRAGGCDIRWLRAQP-LAMGLRNGYRNPDQLGADRWACMVGVLARQPS
                                                    .  .* *   .
```

6C

FIG.~~23C~~

```
B.subtilis|CoaX|SEQIDNO_2               NP--LIVVDFGTATTYCYIDENKQYMGGAIAPGITISTEALYSRAAKLPR
WIT|RCA03301|C.acetobutylicum           RS--LIIIDFGTATTFCAVRENGDYLGGAICPGIKVSSEALFEKAAKLPR
pir|T36391|S.coelicolor                 GP--AIVVDFGTATTFDAVSARGEYIGGVIAPGIEISVEALGVKGAQLRK
sp|O06282|M.tuberculosis                KA--AIVVDFGSSICVDVVSAKGEFLGGAIAPGVQVSSDAAAARSAALRR
WIT|RRC02473|R.capsulatus               GD--LIVVDFGTATTFDVVAPDGAYIGGVIAPGVNLSLEALHMAAAALPH
dbj|BAA21476.1|D.vulgaris               GPRSLVSVDFGTATTFDCVEG-GAYLGGLICPGVLSSAGALSSRTAKLPR
sp|Q9RX54|D.radiodurans                 GLDYAVVVDFGTSTNFDVVGRGRRFLGGILATGAQVSADALFARAAKLPR
gb|AAD35964.1|T.maritima                KN--GIIIDMGTATTVDLVVN-GSYEGGAILPGFFMMVHSLFRGTAKLPL
sp|O83446|T.pallidum                    SA--CVVVDCGTALTFTAVDGTGLIQGVAIAPGLRTAVQSLHTGTAQLPL
sp|O51477|B.burgdorferi                 FEN-VLVVDLGTACTIFAVSRQDGILGGIINSGPLINFNSLLDNAYLIKK
sp|O67753|A.aeolicus                    KN--VVVISAGTALVIDLVLE-GKFKGGFITLGLGKKLKILSDLAEGIPE
sp|P74045|Synechocystis                 FP--CLVVDGGTALTITGFDQDKKLVGGAILPGLGLQLATLGDRLAALPK
sp|O25533|H.pylori                      NG---VVVDAGSAITIDLIKE-GKHLGGCILPGLAQYIHAYKKSAKILEQ
sp|Q45338|B.pertussis                   VHPPLLVASFGTATTLDTIGPDNVFPGGLILPGPAMMRGALAYGTAHLPL
                                              :  . *::      .        *  :  *              :

B.subtilis|CoaX|SEQIDNO_2               IEITRPDN---IIGKNTVSAMQSGILFGYVGQVEGIVKRMKWQAKQDLK-
WIT|RCA03301|C.acetobutylicum           VELIKPAY---AICKNTISSIQSGIVYRYLRQVKYLFEKLKENLPDGRRT
pir|T36391|S.coelicolor                 IEVARPRS---VIGKNTVEAMQSGIVYGFAGQVDGVVNRMARELADD--P
sp|O06282|M.tuberculosis                VELARPRS---VVGKNTVECMQAGAVFGFAGLVDGLVGRIREDVSGFSVD
WIT|RRC02473|R.capsulatus               VDVTKPQG---VIGTNTVACIQSGVYWGYIGLVEGIVRQIRMERDRP---
dbj|BAA21476.1|D.vulgaris               ISLEVEEDS-PVIGRSTTTSLNHGFIFGFAAMTEGVLAA-----------
sp|Q9RX54|D.radiodurans                 ITLQAPET---AIGKNTVHALQSGLVFGYAEMVDGLLRRIRAELPGE---
gb|AAD35964.1|T.maritima                VEVKPADF---VVGKDTEENIRLGVVNGSVYALEGIIGRIKEVYGDLP--
sp|O83446|T.pallidum                    VPLALPDS---VLGKDTTHAVQAGVVRGTLFVIRAMIAQCQKELGCR---
sp|O51477|B.burgdorferi                 FPISTPNN---LLERTTSGSVNSGLFYQYKYLIEGVYRDIKQMYKKK---
sp|O67753|A.aeolicus                    FFPEEVEI---FLGRSTRECVLGGAYRESTEFIKSTLKLWRKVFKRK---
sp|P74045|Synechocystis                 LEMDQLTELPDRWALDTPSAIFSGVVYGVLGALQSYLQDWQKLFPGA---
sp|O25533|H.pylori                      PFKALDSL--EVLPKSTRDAVNYGMVLSVIACIQHLAK--NQK-------
sp|Q45338|B.pertussis                   ADGLVADY-----PIDTHQAIASGIAAAQAGAIVRQWLAGRQRYGQAP--
                                                       *  :  *
```

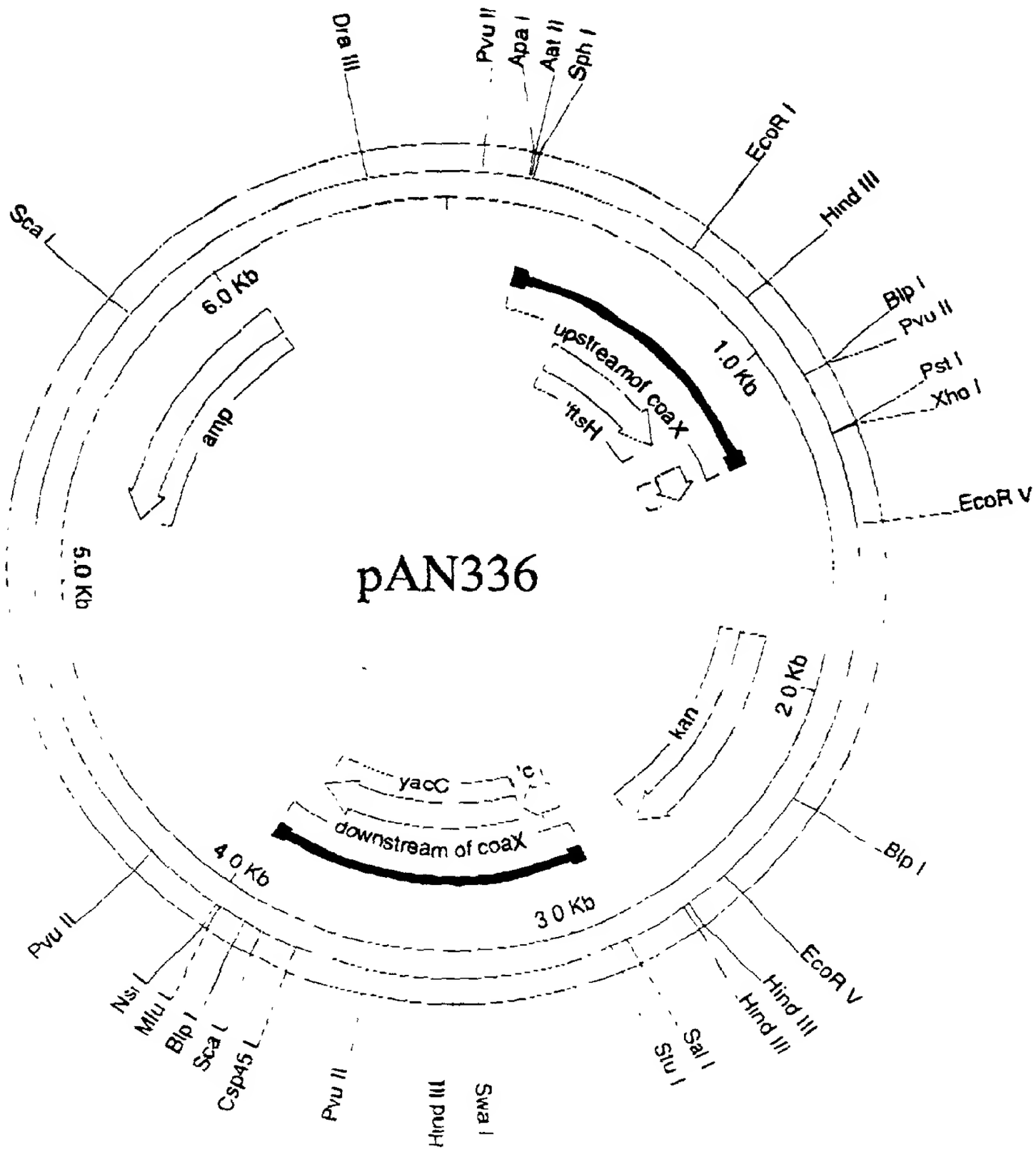
60

FIG. 23D

```
B.subtilis|CoaX|SEQIDNO 2     ----VIATGG--------LAPLIANES-----DCIDIVDPFLTLKGLELI
WIT|RCA03301|C.acetobutylicum RTSLVLATGG--------LAKLIN----------
pir|T36391|S.coelicolor       DDVTVIATGG--------LAPMVLGES-----SVIDEHEPWLTLMGLRLV
sp|O06282|M.tuberculosis      HDVAIVATGH--------TAPLLLPEL-----HTVDHYDQHLTLQGLRLV
WIT|RRC02473|R.capsulatus     --MKVIATGG--------LASLFDLGF-----DLFDKVEDDLTMHGLRLI
dbj|BAA21476.1|D.vulgaris     --------------------------------------------------
sp|Q9RX54|D.radiodurans       --AVAVATGG--------FSRTVQGIC-----QEIDYYDETLTLRGLVEL
gb|AAD35964.1|T.maritima      ----VVLTGG--------QSKIVK-DM-----IKHEIFDEDLTIKGVYHF
sp|O83446|T.pallidum          --CAAVITGG--------LSRLFS-SE-----VDFPPIDAQLTLSGLAHI
sp|O51477|B.burgdorferi       --FNLIITGG--------NADLILSLI-----EIEFIFNIHLTVEGVRIL
sp|O67753|A.aeolicus          --FKVVITGG--------EGKYFS---------KFGIYDPLLVHRGMRNL
sp|P74045|Synechocystis       ---AMVITGG--------DGKILHGFLKEHSPNLSVAWDDNLIFLGMAAI
sp|O25533|H.pylori            ----IYLCGG--------DAKYLSAFL------PHSVCKERLVFDGMEIA
sp|Q45338|B.pertussis         ---EIYVAGGGWPEVRQEAERLLAVTGAAFGATPQPTYLDSPVLDGLAAL
```

```
B.subtilis|CoaX|SEQIDNO 2     YERNRVGSV----------
WIT|RCA03301|C.acetobutylicum ----------
pir|T36391|S.coelicolor       YERNVSRM-----------
sp|O06282|M.tuberculosis      FERNLEVQRGRLKTAR----
WIT|RRC02473|R.capsulatus     FDYNKGLGA----------
dbj|BAA21476.1|D.vulgaris     ----------
sp|Q9RX54|D.radiodurans       WASRSEVR-----------
gb|AAD35964.1|T.maritima      CFGD---------------
sp|O83446|T.pallidum          ARLVPTSLLPPATVSGSSGN
sp|O51477|B.burgdorferi       GNSIDFKFVN---------
sp|O67753|A.aeolicus          LYLYHRI------------
sp|P74045|Synechocystis       HHGDRPIC-----------
sp|O25533|H.pylori            LKKAGILECK---------
sp|Q45338|B.pertussis         AAQGAPTA-----------
```

Figure 2: Structure of pAN336, a plasmid designed to delete B. subtilis coaX *from the chromosome and replace it with a kanamycin resistance gene.*



Construction of pOTP72

Fig 8

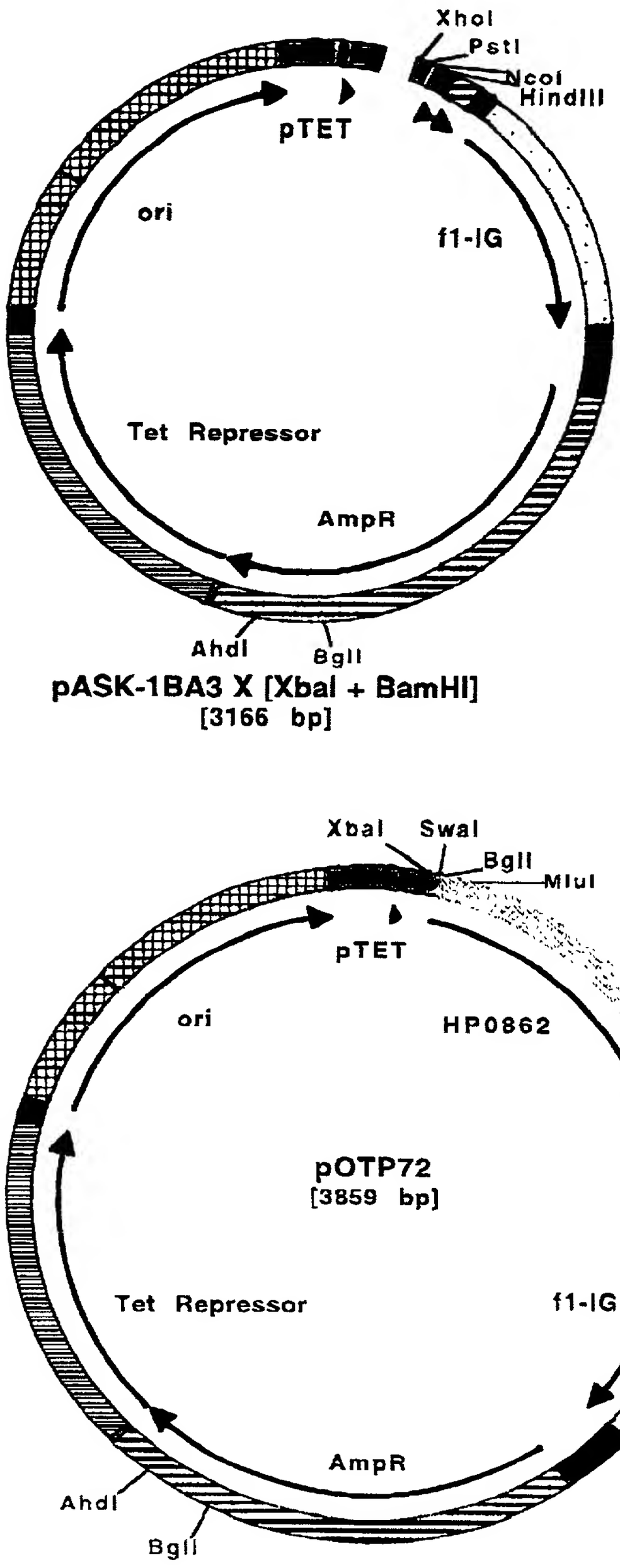
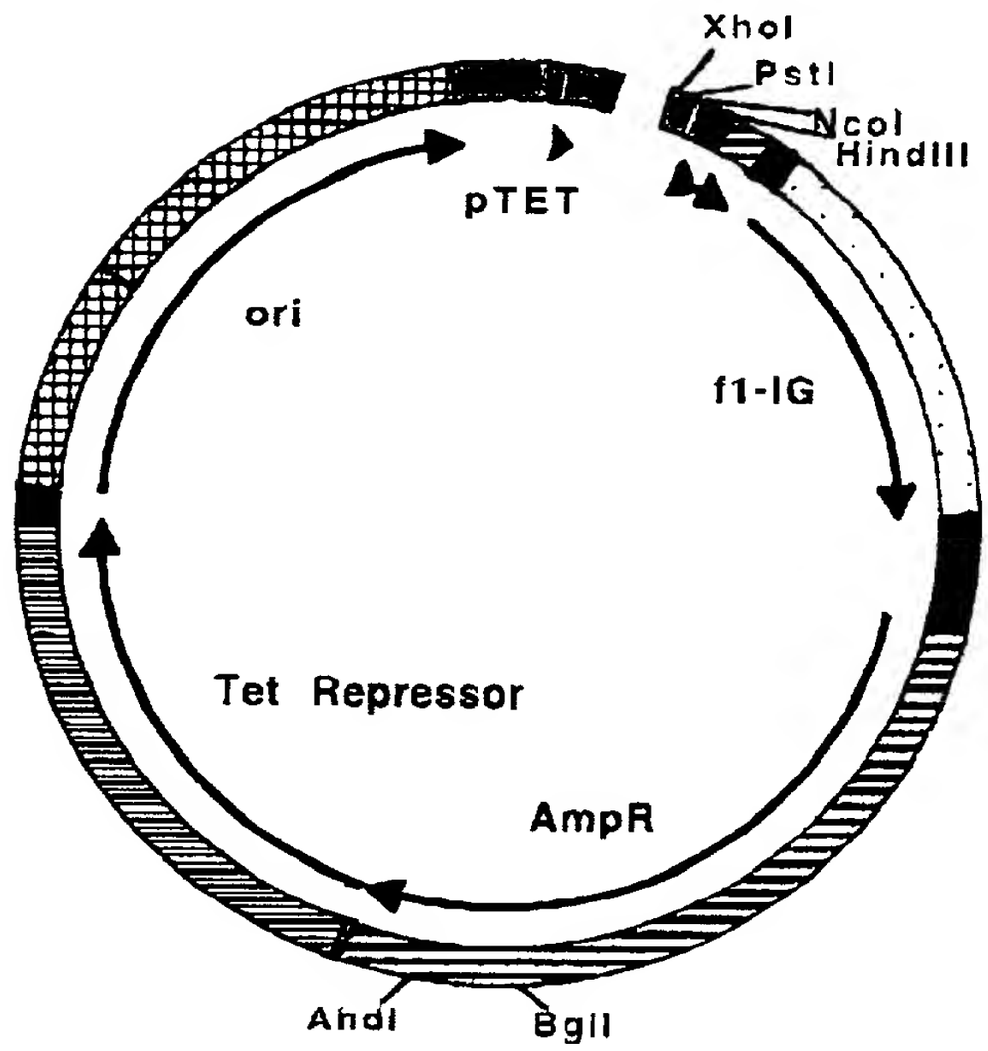
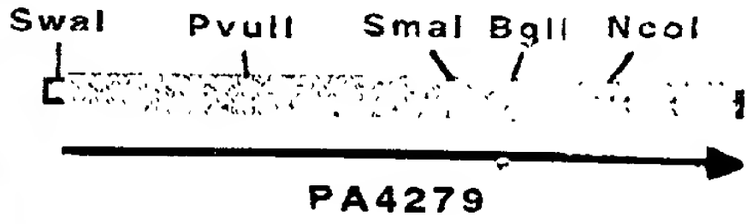


Fig 9

Construction of pOTP73



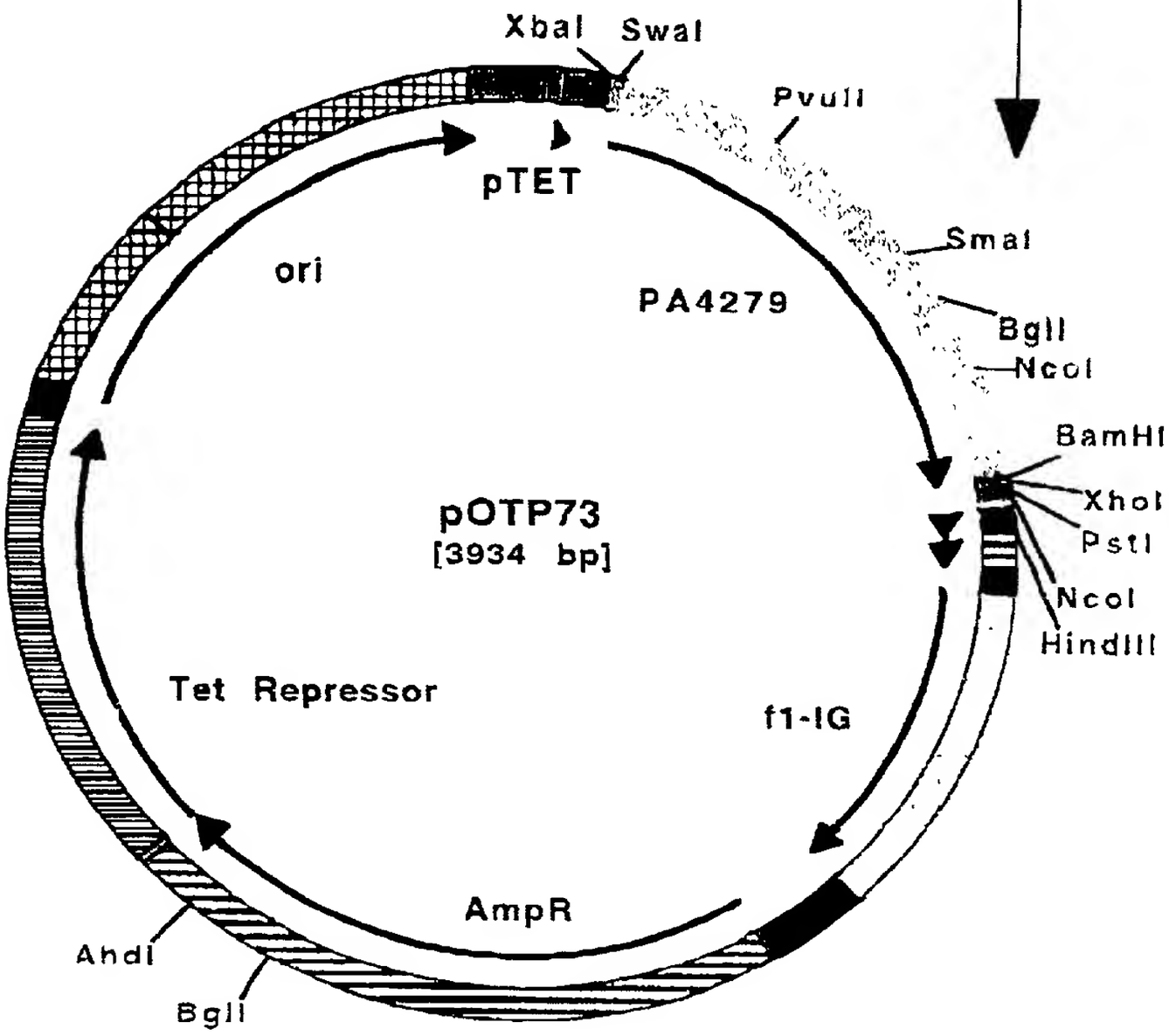
pASK-1BA3 X [XbaI + BamHI]
[3166 bp]



P. aeruginosa coaX (PA4279)
PCR product X [XbaI + BamHI]
[768 bp]

PA4279

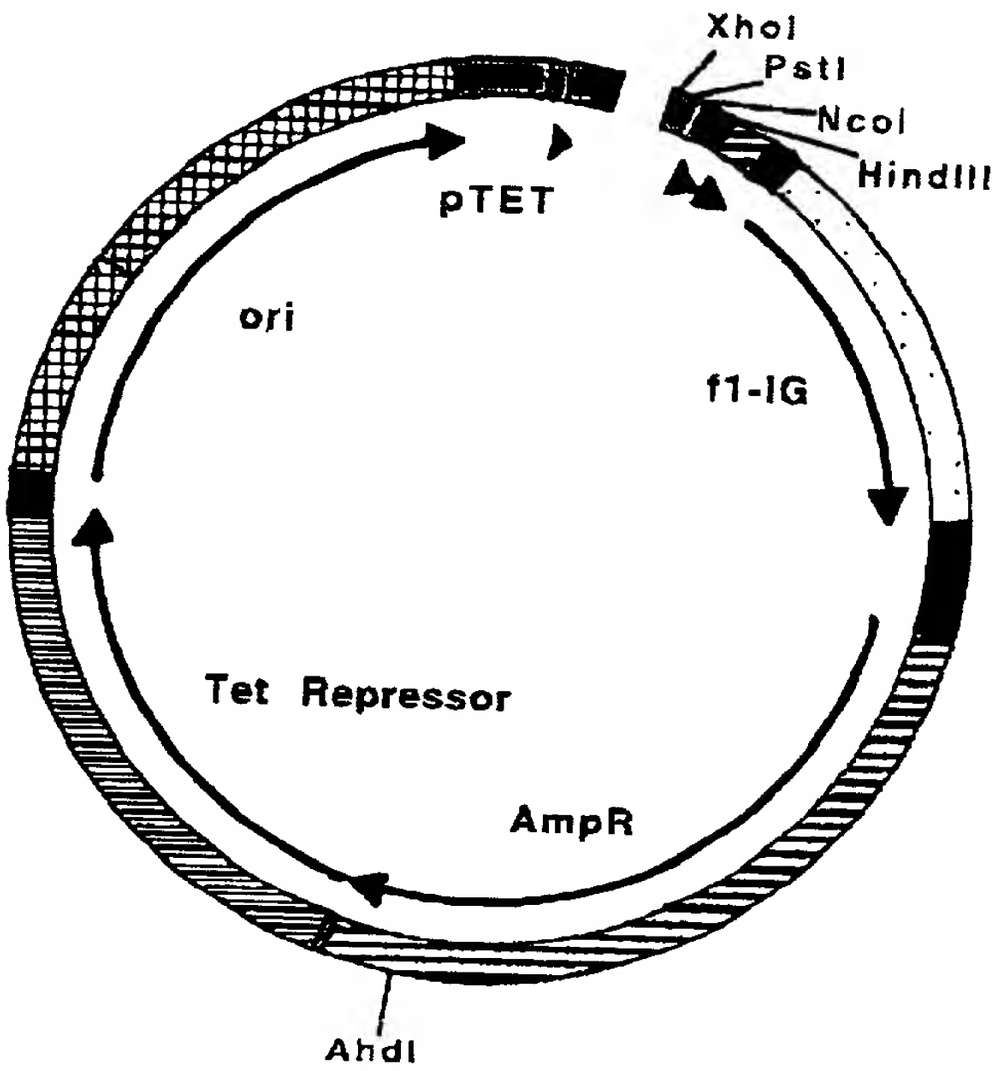
T4 DNA ligase



pOTP73
[3934 bp]

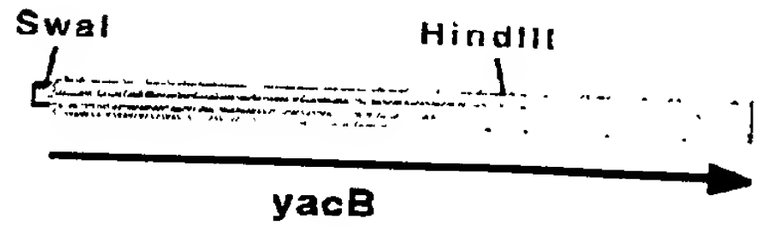
Fig 10

Construction of pOTP71

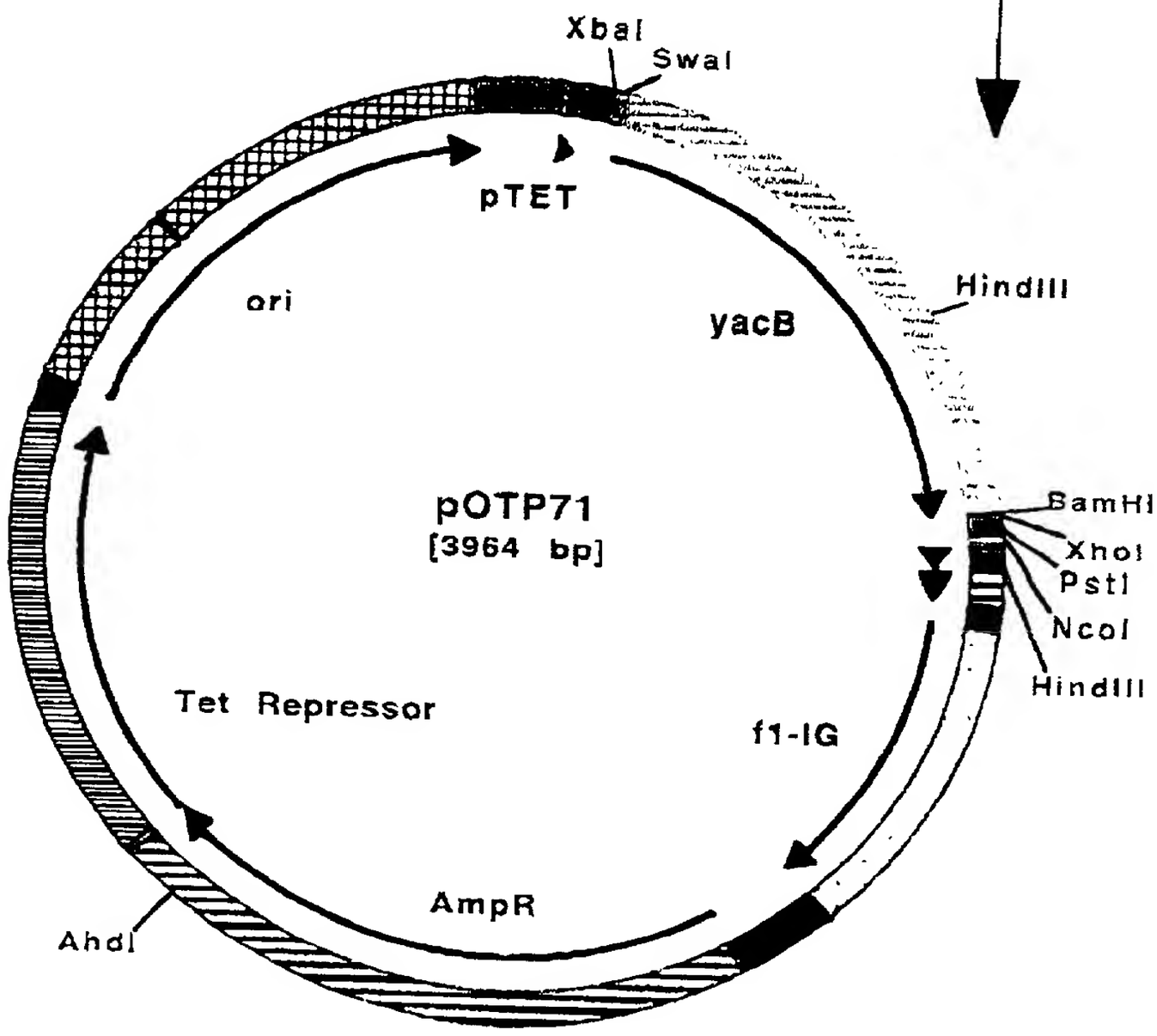


pASK-1BA3 X [XbaI + BamHI]
[3166 bp]

B. subtilis coaX (yacB) PCR product X [XbaI + BamHI]
[798 bp]



T4 DNA ligase



pOTP71
[3964 bp]